Clinical Biochemistry 49 (2016) 139–149

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Transference of CALIPER pediatric reference intervals to biochemical assays on the Roche cobas 6000 and the Roche Modular P

Victoria Higgins [a,b], Man Khun Chan [a], Michelle Nieuwesteeg [a], Barry R. Hoffman [c], Irvin L. Bromberg [c], Doug Gornall [d], Edward Randell [e], Khosrow Adeli [a,b,*]

[a] CALIPER Program, Pediatric Laboratory Medicine, The Hospital for Sick Children, Canada
[b] Department of Laboratory Medicine & Pathobiology, University of Toronto, Toronto, ON, Canada
[c] Laboratory Medicine and Pathobiology, Mount Sinai Hospital, Toronto, ON, Canada
[d] Department of Pathology, Toronto East General Hospital, Toronto, ON, Canada
[e] Eastern Health, St. John's, NL, Canada

ARTICLE INFO

Article history:
Received 8 June 2015
Received in revised form 24 July 2015
Accepted 17 August 2015
Available online 19 August 2015

Keywords:
Reference intervals
Abbott Architect
Roche cobas 6000
Roche Modular P
CALIPER
Pediatrics

ABSTRACT

Objectives: The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) has recently established pediatric age- and sex-specific reference intervals for over 85 biochemical markers on the Abbott Architect system. Previously, CALIPER reference intervals for several biochemical markers were successfully transferred from Abbott assays to Roche, Beckman, Ortho, and Siemens assays. This study further broadens the CALIPER database by performing transference and verification for 52 biochemical assays on the Roche cobas 6000 and the Roche Modular P.

Design and methods: Using CLSI C28-A3 and EP9-A2 guidelines, transference of the CALIPER reference intervals was attempted for 16 assays on the Roche cobas 6000 and 36 on the Modular P. Calculated reference intervals were further verified using 100 healthy CALIPER samples.

Results: Most assays showed strong correlation between assay systems and were transferable from Abbott to the Roche cobas 6000 (81%) and the Modular P (86%). Bicarbonate and magnesium were not transferable on either system and calcium and prealbumin were not transferable to the Modular P. Of the transferable analytes, 62% and 61% were verified on the cobas 6000 and the Modular P, respectively.

Conclusions: This study extends the utility of the CALIPER database to two additional analytical systems, which facilitates the broad application of CALIPER reference intervals at pediatric centers utilizing Roche biochemical assays. Transference studies across different analytical platforms can later be collectively analyzed in an attempt to develop common reference intervals across all clinical chemistry instruments to harmonize laboratory test interpretation in diagnosis and monitoring of pediatric disease.

1. Introduction

Accurate clinical interpretation of laboratory test results requires appropriately partitioned reference intervals. By definition, reference intervals encompass the central 95% of the distribution of test values from a healthy reference population [1]; thus, test values that lie outside of this interval are considered abnormal, indicating further testing and/or appropriate treatment are required. Unfortunately, there remain major gaps in pediatric reference data. Analyte levels can vary dramatically with growth and development, and therefore pediatric reference intervals require consideration of key covariates such as age and sex [2,3]. Additional obstacles for establishing reference intervals for pediatrics include attaining parental consent, recruiting a sufficient number of healthy young children, and obtaining sufficient blood sample volumes [4].

The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER), a collaborative project among several pediatric centers across Canada, has been addressing the critical gaps in pediatric reference intervals [4]. To date, CALIPER has collected over 8500 blood samples from healthy children to establish robust sex- and age-stratified pediatric reference intervals for over 85 biochemical markers including serum chemistry, hormone, enzyme, lipid, lipoprotein, and protein

Abbreviations: ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; Albumin BCP, Albumin bromcresol purple; Albumin BCG, Albumin bromcresol green; ApoB, Apolipoprotein B; ASO, Anti-streptolysin-O; AST, Aspartate aminotransferase; CALIPER, Canadian Laboratory Initiative on Pediatric Reference Intervals; CLSI, Clinical Laboratory Standards Institute; HCO_3^-, bicarbonate; CRP, C-reactive protein; C3, Complement component 3; C4, Complement component 4; GGT, Gamma-glutamyl transferase; HDLC, High-density lipoprotein cholesterol; hsCRP, High sensitivity C-reactive protein; IgA, Immunoglobulin A; IgG, Immunoglobulin G; IgM, Immunoglobulin M; LD, Lactate dehydrogenase; RF, Rheumatoid factor; RMSE, Root mean-squared error.

* Corresponding author at: Clinical Biochemistry, Department of Pediatric Laboratory Medicine, The Hospital for Sick Children, 555 University Avenue, Toronto, ON M5G 1X8, Canada.
E-mail address: khosrow.adeli@sickkids.ca (K. Adeli).

http://dx.doi.org/10.1016/j.clinbiochem.2015.08.018
0009-9120/

markers [2,5,6]. CALIPER's goal is to establish an up-to-date database of pediatric reference values that can be used to interpret test results from all major clinical chemistry analyzers used in Canadian laboratories. Initially, CALIPER measured most analytes using the Abbott Architect system; thus, reference intervals were only applicable to laboratories using this analytical platform. Performing de novo reference interval studies for each of the major analytical systems used in Canadian laboratories would be impractical due to the burden of costs and logistics. Therefore, CALIPER has initiated a series of transference studies using Clinical Laboratory Standards Institute (CLSI) guidelines [1], in an effort to transfer CALIPER reference intervals established on the Abbott Architect to biochemical assays on other common analytical platforms, including the Beckman Coulter DxC800, Ortho Vitros 5600, Roche cobas 6000, and Siemens Vista 1500 [7].

In order to broaden the CALIPER database, we report here transference of reference intervals for 16 assays to the Roche cobas 6000 and 36 assays on the Roche Modular P. Currently, reference interval studies on both the Roche cobas [8] and the Roche Modular P are very limited [9]. This study will establish assay-specific, age- and sex-stratified pediatric reference intervals for a large number of biochemical markers, which will globally benefit clinical laboratories using Roche biochemical assays.

2. Materials and methods

2.1. Method comparison sample analysis

This study was approved by the Institutional Review Board (IRB) at the Hospital for Sick Children (Toronto, Canada) and the review boards of collaborating hospitals. Approximately 200 pooled serum specimens, each containing serum from 3–4 pediatric patients attending The Hospital for Sick Children (Toronto, Ontario), were analyzed on the Roche cobas 6000 (at Toronto East General Hospital, Toronto, Ontario), the Roche Modular P (at Mount Sinai Hospital, Toronto, Ontario), and the Abbott Architect ci8200 (Eastern Health, St. John's, Newfoundland). Samples were selected to ensure that a broad concentration/activity range would be covered for each analyte under study. Chemistry assays measured include: direct bilirubin, total bilirubin, total calcium, bicarbonate (HCO_3^-), creatinine, magnesium, iron, inorganic phosphate, urea, and uric acid. Enzymes measured include: alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and lactate dehydrogenase (LD). Lipid/lipoproteins measured include: apolipoprotein B (apoB), cholesterol, high-density lipoprotein cholesterol (HDLC), and total triglycerides. Lastly, proteins measured include: albumin, complement component 3 (C3), complement component 4 (C4), C-reactive protein (CRP), high sensitivity C-reactive protein (hsCRP), haptoglobin, immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), prealbumin, total protein, anti-streptolysin O (ASO), rheumatoid factor (RF), and transferrin. Not all analytes were tested on all instruments, as reference intervals for 19 other assays on the Roche cobas 6000 have previously been transferred [7]. A complete list of biochemical markers analyzed on each Roche instrument and analytical performance specifications for assays on all three analytical instruments are summarized in Supplemental Tables 1–3.

2.2. Transference protocols and statistical analysis

Supplemental Fig. 1 provides a summary of the data analysis procedure and the criteria for transference and verification according to the CLSI C28-A3 and EP9-A2 guidelines [1,10]. Statistical analysis was performed using Excel (Microsoft) and R software [11]. Analyte concentrations obtained from each Abbott Architect assay were plotted against the corresponding concentrations obtained from each of the Roche instruments under investigation. Gross outliers were removed by visual examination and results below the lower end of the reportable range were excluded. In addition, isolated extreme high and low data points along the regression line were removed (based on CLSI guidelines) in order to avoid overestimation of the quality of correlation [1,10]. If the data yielded an $R^2 \geq 0.95$, simple linear regression using the least squares approach was used to determine the line of best fit. If $0.70 < R^2 < 0.95$, Deming regression was used to determine the line of best fit. If $R^2 < 0.70$, it was concluded that the correlation between data from the two systems was inadequate and the reference intervals could not be reliably transferred.

To assess the appropriateness of a linear model with normally distributed data points, quantile–quantile (Q–Q), studentized residual, and Bland–Altman plots were generated for each assay. The latter two plots were visually examined to confirm that no distinct patterns were observed in the data points, indicating random distribution of the residuals and lack of any biased trends in the relationship between the two methods. Q–Q plots show the distance between a point and the regression line (i.e. the studentized residual) on the y-axis, as a function of what that distance would be if the residuals were normally distributed (i.e. the theoretical quantile) on the x-axis. Therefore, this plot was used to determine whether the residuals followed a normal distribution, observed as a straight line of the equation $y = x$. If the criteria of these 3 graphs were met, the equation of the line of best fit was used to transfer the CALIPER reference intervals established using the Abbott Architect to the Roche cobas 6000 or the Modular P. It is important to note that an analyte was considered non-transferable in cases where one of the graphs indicated biased or non-normal distribution of the residuals, even if a strong correlation was observed. The root of the mean-squared error (RMSE) was used to determine 95% confidence intervals around each reference limit, calculated as the reference limit $\pm$ $1.96 * \text{RMSE}$. The confidence intervals were used as limits with which to verify the transferred reference intervals.

2.3. Verification of transferred reference intervals using samples from the CALIPER cohort

Although CLSI C28-A3 guidelines require only 20 reference samples for verification of transferred reference intervals [1], in the current study, approximately 100 reference specimens from the CALIPER biobank of healthy pediatric samples were analyzed on the Roche cobas 6000 and the Modular P. Specimens were selected to encompass as many age and sex partitions as possible. Reference intervals were considered verified when >90% of the reference samples fell within the transferred reference intervals, inclusive of the 95% confidence intervals.

3. Results

Approximately 200 pooled serum specimens were used to determine the relationship between the Abbott Architect assays and those from the Roche cobas 6000 (16 analytes) or the Modular P (33 analytes). Of note, the Roche Modular P has multiple tests for some analytes, which employ different reagents and methodologies, whereas reference intervals were only established for one corresponding test on the Abbott Architect. Firstly, ALT was measured on the Abbott Architect, while both ALT and ALT pyridoxal phosphate (ALT PP) were measured on the Modular P. Secondly, AST was measured on Abbott, while both AST and AST PP were measured on the Modular P. Lastly, albumin bromcresol purple (BCP) was measured on Abbott, while both albumin bromcresol green (BCG) and albumin bromcresol purple (BCP) were measured on the Modular P. For these analytes, the reference intervals determined for the Abbott test were compared to both Roche methods. Thus, a total of 16 assays were examined on the cobas 6000 and 36 assays (33 analytes) were examined on the Modular P. The majority of reference intervals established on the Abbott Architect transferred to both Roche instruments, with 81% (13 of 16) assays successfully transferring

to the cobas 6000 and 86% (31 of 36) assays successfully transferring to the Modular P.

3.1. Transference of reference intervals

Scatter plots and corresponding correlation coefficients were assessed. In the majority of cases, a linear relationship was observed suggesting that results from the Roche assays correlated well with values obtained for the corresponding Abbott Architect assays. A representative scatter plot to assess transferability between the Abbott Architect and Roche cobas 6000 is shown in Fig. 1A for transferrin ($R^2 = 0.924$), which demonstrated a strong correlation. The R^2 values for all analytes are reported on the scatter plots in Supplemental Figs. 2–5. Among the transferable analytes, the R^2 values ranged from 0.78–1.0 across both Roche systems. Simple linear regression using the least squares approach was used to calculate the equation of best fit line for R^2 values ≥0.95. This was the case for amylase, GGT and creatinine on both Roche instruments, for triglycerides, direct bilirubin, hsCRP and C4 on the cobas 6000, and for ALP, ALT, ALT PP, AST, AST PP, CRP, haptoglobin, total bilirubin and IgG on the Modular P. The equation of best fit line was calculated using Deming regression when $0.70 < R^2 < 0.95$, which was the case for albumin, apoB, HDLC, ASO, C3, and transferrin on both Roche instruments, and for cholesterol, IgA, IgM, iron, LD, phosphate, total protein, urea, triglycerides, uric acid, direct bilirubin, and C4 on the Modular P. Two notable exceptions were bicarbonate and magnesium, for which poor correlation was found between the Abbott Architect and both Roche assays, where R^2 values ranged between 0.23–0.3 for bicarbonate and 0.67–0.69 for magnesium (Supplemental Figs. 3A, B, 5A, B). Therefore, bicarbonate and magnesium reference intervals were deemed not transferrable. For the Modular P, total calcium ($R^2 = 0.53$) and prealbumin ($R^2 = 0.27$) reference intervals were also not transferred due to poor correlation. As expected for a healthy population, the vast majority of RF values were below the detectable limit of the Abbott Architect (15.0 IU/mL), Roche cobas 6000 (10 IU/mL) and Roche Modular P (6.6 IU/mL) assays.

Of the 16 assays measured on the cobas 6000 and the 36 measured on the Modular P, 13 and 31 of these correlated well with the Abbott Architect ($R^2 > 0.70$), respectively. These analytes were then subjected to further statistical analysis to assess the appropriateness of transference. Equal scatter around the best fit line, normal distribution of residuals, and the absence of data clustering were assessed by generating and visually analyzing QQ plots, Bland–Altman, and studentized residual plots. In most instances, the QQ plot generated a straight line, indicative of normality, the residuals were randomly distributed, and the Bland–Altman plot did not show discrete groups of data points (example shown in Fig. 1B–D for transferrin). In these cases, the regression equations were used to transfer the Abbott reference intervals to the Roche cobas 6000 and/or Modular P. Table 1 displays transferred Roche reference intervals for the cobas 6000, and Table 2 shows reference intervals for the Modular P. Notably, for ALT, the Abbott reference intervals were transferable to both assays on the Modular P, ALT and ALT PP. The same was true for AST and AST PP, as well as for albumin BCG and albumin BCP. Furthermore, the Abbott hsCRP assay was compared to the conventional CRP assay on the Modular P and reference intervals were

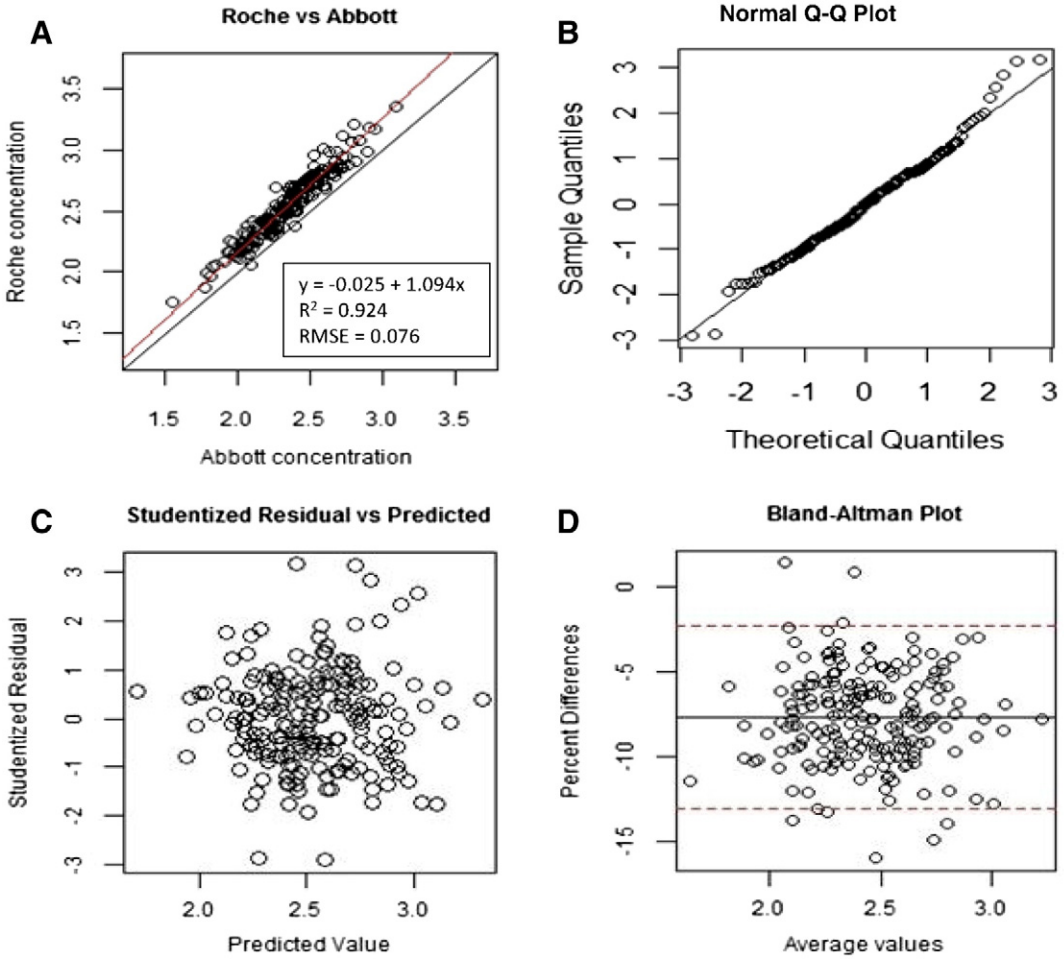

Fig. 1. Statistical criteria used to assess the appropriateness of transference. Representative scatter (A), Q–Q (B), studentized residual (C) and Bland–Altman (D) plots for analytes where transference was deemed appropriate (transferrin on Roche cobas 6000, A–D). In the scatterplot (A), the line of perfect agreement is shown in black, while the regression line is shown in red.

Table 1
Age- and sex-specific pediatric reference intervals for transferred assays from Abbott Architect to Roche cobas 6000.[a]

Analyte	Age	Female Reference Interval				Male Reference Interval			
		Lower Limit	Upper Limit	Lower Limit Confidence Interval	Upper Limit Confidence Interval	Lower Limit	Upper Limit	Lower Limit Confidence Interval	Upper Limit Confidence Interval
Chemistry									
Bilirubin, direct (μmol/L)	0 - 14 d	4.3	9.4	(3.5 , 5.0)	(8.6 , 10.1)	4.3	9.4	(3.5 , 5.0)	(8.6 , 10.1)
	15 d - < 1 yr	<2.0	3.9	(<2.0[c] , <2.0)	(3.1 , 4.6)	<2.0	3.9	(<2.0[c] , <2.0)	(3.1 , 4.6)
	1 - < 9 yrs	<2.0	2.4	(<2.0 , <2.0)	(<2.0 , 3.2)	<2.0	2.4	(<2.0 , <2.0)	(<2.0 , 3.2)
	9 - < 13 yrs	<2.0	3.7	(<2.0 , <2.0)	(3.0 , 4.4)	<2.0	3.7	(<2.0 , <2.0)	(3.0 , 4.4)
	13 - < 19 yrs	<2.0	5.1	(<2.0 , <2.0)	(4.3 , 5.8)	<2.0	5.4	(<2.0 , <2.0)	(4.6 , 6.1)
Creatinine (enzymatic) (μmol/L)	0 - 14 d	35	86	(30 , 39)	(82 , 91)	35	86	(30 , 39)	(82 , 91)
	15 d - < 2 yrs	15	38	(10 , 20)	(33 , 42)	15	38	(10 , 20)	(33 , 42)
	2 - < 5 yrs	24	43	(19 , 29)	(39 , 48)	24	43	(19 , 29)	(39 , 48)
	5 - < 12 yrs	33	59	(28 , 37)	(54 , 64)	33	59	(28 , 37)	(54 , 64)
	12 - < 15 yrs	45	77	(41 , 50)	(72 , 81)	45	77	(41 , 50)	(72 , 81)
	15 - < 19 yrs	48	79	(43 , 53)	(74 , 83)	60	100	(55 , 65)	(95 , 105)
Enzymes									
Amylase (U/L)	0 - 14 d	3	11	(<3 , 7)	(7 , 16)	3	11	(<3 , 7)	(7 , 16)
	15d -<13wks	<3	26	(<3 , 6)	(21 , 30)	<3	26	(<3 , 6)	(21 , 30)
	13 wks -<1yr	3	58	(<3 , 7)	(54 , 62)	3	58	(<3 , 7)	(54 , 62)
	1 - < 19 yrs	29	118	(25 , 33)	(114 , 122)	29	118	(25 , 33)	(114 , 122)
GGT[b] (U/L)	0 - 14 d	17	175	(14 , 21)	(172 , 178)	17	175	(14 , 21)	(172 , 178)
	15 d - < 1 yr	5	101	(2 , 8)	(98 , 104)	5	101	(2 , 8)	(98 , 104)
	1 - < 11 yrs	4	12	(0 , 7)	(8 , 15)	4	12	(0 , 7)	(8 , 15)
	11 - < 19 yrs	4	16	(1 , 8)	(12 , 19)	4	16	(1 , 8)	(12 , 19)
Proteins									
Albumin BCP	0 - 14 d	34	47	(31 , 36)	(45 , 50)	34	47	(31 , 36)	(45 , 50)
	15 d - < 1 yr	30	53	(28 , 33)	(50 , 55)	30	53	(28 , 33)	(50 , 55)
	1 - < 8 yrs	41	52	(39 , 43)	(49 , 54)	41	52	(39 , 43)	(49 , 54)
	8 - < 15 yrs	43	54	(41 , 46)	(51 , 56)	43	54	(41 , 46)	(51 , 56)
	15 - < 19 yrs	41	56	(39 , 43)	(53 , 58)	44	57	(42 , 47)	(55 , 59)
ASO (IU/mL)	0 - < 6 mths	<20[c]	<20	(<20 , <20)	(<20 , <20)	<20	<20	(<20 , <20)	(<20 , <20)
	6 mths -<1yr	<20	<20	(<20 , <20)	(<20 , 60)	<20	<20	(<20 , <20)	(<20 , 60)
	1 - < 6 yrs	<20	147	(<20 , <20)	(106 , 187)	<20	147	(<20 , <20)	(106 , 187)
	6 - < 19 yrs	<20	535	(<20 , <20)	(495 , 576)	<20	535	(<20 , <20)	(495 , 576)
C3 (g/L)	0 - 14 d	0.57	1.29	(0.49 , 0.65)	(1.21 , 1.37)	0.57	1.29	(0.49 , 0.65)	(1.21 , 1.37)
	15 d - < 1 yr	0.58	1.69	(0.50 , 0.66)	(1.61 , 1.77)	0.58	1.69	(0.50 , 0.66)	(1.61 , 1.77)
	1 - < 19 yrs	0.90	1.61	(0.82 , 0.98)	(1.53 , 1.69)	0.90	1.61	(0.82 , 0.98)	(1.53 , 1.69)
C4 (g/L)	0 - < 1 yr	0.07	0.31	(0.05 , 0.09)	(0.29 , 0.33)	0.07	0.31	(0.05 , 0.09)	(0.29 , 0.33)
	1 - < 19 yrs	0.13	0.38	(0.11 , 0.15)	(0.36 , 0.4)	0.13	0.38	(0.11 , 0.15)	(0.36 , 0.4)
hsCRP (mg/L)	0 – 14 d	0.6	6.1	(0 , 1.8)	(5.0 , 7.3)	0.6	6.1	(0 , 1.8)	(5.0 , 7.3)
	15 d – 1 yr	0.4	1.3	(0 , 1.6)	(0.1 , 2.5)	0.4	1.3	(0 , 1.6)	(0.1 , 2.5)
	15 - <19 yrs	0.4	2.0	(0 , 1.6)	(0.8 , 3.1)	0.4	2.0	(0 , 1.6)	(0.8 , 3.1)
Transferrin (g/L)	0 - < 9 wks	1.11	2.43	(0.96 , 1.26)	(2.28 , 2.58)	1.11	2.43	(0.96 , 1.26)	(2.28 , 2.58)
	9 wks - < 1 yr	1.15	3.52	(1.00 , 1.30)	(3.37 , 3.67)	1.15	3.52	(1.00 , 1.30)	(3.37 , 3.67)
	1 - < 19 yrs	2.38	3.66	(2.23 , 2.53)	(3.51 , 3.81)	2.38	3.66	(2.23 , 2.53)	(3.51 , 3.81)
Lipids/Lipoproteins									
ApoB (g/L)	0 - 14 d	0.1	0.7	(0.0 , 0.1)	(0.6 , 0.7)	0.1	0.7	(0.0 , 0.1)	(0.6 , 0.7)
	15 d - < 1 yr	0.2	1.3	(0.1 , 0.2)	(1.2 , 1.3)	0.2	1.3	(0.1 , 0.2)	(1.2 , 1.3)
	1 - < 6 yrs	0.4	0.9	(0.4 , 0.5)	(0.9 , 1.0)	0.4	0.9	(0.4 , 0.5)	(0.9 , 1.0)
	6 - < 19 yrs	0.3	0.9	(0.2 , 0.3)	(0.8 , 0.9)	0.3	0.9	(0.2 , 0.3)	(0.8 , 0.9)
HDLC (mmol/L)	0 - 14 d	0.18	1.01	(<0.08 , 0.31)	(0.88 , 1.14)	0.18	1.01	(<0.08 , 0.31)	(0.88 , 1.14)
	15 d - < 1 yr	<0.08	1.95	(<0.08 , 0.19)	(1.82 , 2.08)	<0.08	1.95	(<0.08 , 0.19)	(1.82 , 2.08)
	1 - < 4 yrs	0.72	1.68	(0.59 , 0.85)	(1.55 , 1.81)	0.72	1.68	(0.59 , 0.85)	(1.55 , 1.81)
	4 - < 13 yrs	0.81	1.99	(0.68 , 0.94)	(1.86 , 2.12)	0.81	1.99	(0.68 , 0.94)	(1.86 , 2.12)
	13 - < 19 yrs	0.70	1.96	(0.57 , 0.83)	(1.83 , 2.09)	0.69	1.85	(0.56 , 0.82)	(1.72 , 1.98)
Triglycerides (mmol/L)	0 - 14 d	1.02	3.25	(0.92 , 1.12)	(3.15 , 3.35)	1.02	3.25	(0.92 , 1.12)	(3.15 , 3.35)
	15 d - < 1 yr	0.65	3.24	(0.55 , 0.75)	(3.14 , 3.34)	0.65	3.24	(0.55 , 0.75)	(3.14 , 3.34)
	1 - < 19 yrs	0.54	2.47	(0.44 , 0.64)	(2.37 , 2.57)	0.54	2.47	(0.44 , 0.64)	(2.37 , 2.57)

[a]Partitions requiring sex partitioning are underlined. Female reference intervals are highlighted in pink, whereas male reference intervals are highlighted in blue.

[b]GGT, gamma-glutamyl transferase; Albumin BCP, albumin bromcresol purple; ASO, anti-streptolysin O; C3, complement C3; C4, complement C4; hsCRP, high sensitivity C-reactive protein; ApoB, apolipoprotein B; HDLC, high-density lipoprotein cholesterol.

[c]'<' indicates values lower than the detection limit

Table 2
Age- and sex-specific pediatric reference intervals for transferred assays from Abbott Architect to the Roche Modular P.[a]

Analyte	Age	Female Reference Interval				Male Reference Interval			
		Lower Limit	Upper Limit	Lower Limit Confidence Interval	Upper Limit Confidence Interval	Lower Limit	Upper Limit	Lower Limit Confidence Interval	Upper Limit Confidence Interval
Chemistry									
Bilirubin, direct (umol/L)	0 - 14 d	3.4	7.7	(2.4 , 4.3)	(6.7 , 8.6)	3.4	7.7	(2.4 , 4.3)	(6.7 , 8.6)
	15 d - < 1 yr	<1.2	3.0	(<1.2 , <1.2)	(2.1 , 4.0)	<1.2	3.0	(<1.2 , <1.2)	(2.1 , 4.0)
	1 - < 9 yrs	<1.2	1.8	(<1.2 , <1.2)	(<1.2 , 2.8)	<1.2	1.8	(<1.2 , <1.2)	(<1.2 , 2.8)
	9 - < 13 yrs	<1.2	2.9	(<1.2 , <1.2)	(2.0 , 3.9)	<1.2	2.9	(<1.2 , <1.2)	(2.0 , 3.9)
	13 - < 19 yrs	<1.2	4.0	(<1.2 , 1.6)	(3.1 , 5.0)	<1.2	4.3	(<1.2 , 1.8)	(3.4 , 5.3)
Bilirubin, total (umol/L)	0 - 14 d	2.4	247.0	(<1.2 , 4.9)	(244.4 , 249.5)	2.4	247.0	(<1.2 , 4.9)	(244.4 , 249.5)
	15 d - < 1 yr	<1.2	9.7	(<1.2 , 2.7)	(7.1 , 12.2)	<1.2	9.7	(<1.2 , 2.7)	(7.1 , 12.2)
	1 - < 9 yrs	<1.2	5.4	(<1.2 , 2.7)	(2.9 , 8.0)	<1.2	5.4	(<1.2 , 2.7)	(2.9 , 8.0)
	9 - < 12 yrs	<1.2	7.7	(<1.2 , 2.7)	(5.1 , 10.2)	<1.2	7.7	(<1.2 , 2.7)	(5.1 , 10.2)
	12 - < 15 yrs	<1.2	9.9	(<1.2 , 3.5)	(7.3 , 12.4)	<1.2	9.9	(<1.2 , 3.5)	(7.3 , 12.4)
	15 - < 19 yrs	1.0	12.0	(<1.2 , 3.5)	(9.5 , 14.6)	1.0	12.0	(<1.2 , 3.5)	(9.5 , 14.6)
Creatinine (enzymatic) (umol/L)	0 - 14 d	27	79	(21 , 33)	(73 , 85)	27	79	(21 , 33)	(73 , 85)
	15 d - < 2yrs	8	30	(1 , 14)	(24 , 36)	8	30	(1 , 14)	(24 , 36)
	2 - < 5 yrs	16	36	(10 , 23)	(30 , 42)	16	36	(10 , 23)	(30 , 42)
	5 - < 12 yrs	25	52	(19 , 32)	(45 , 58)	25	52	(19 , 32)	(45 , 58)
	12 - < 15 yrs	38	69	(32 , 44)	(63 , 75)	38	69	(32 , 44)	(63 , 75)
	15 - < 19 yrs	41	71	(34 , 47)	(65 , 77)	53	93	(46 , 59)	(86 , 99)
Iron (umol/L)	0 - < 14 yrs	3	24	(1 , 5)	(22 , 26)	3	24	(1 , 5)	(22 , 26)
	14 - < 19 yrs	4	30	(2 , 6)	(28 , 32)	6	31	(4 , 8)	(29 , 33)
Phosphate (mmol/L)	0 - 14 d	1.82	3.45	(1.76 , 1.88)	(3.39 , 3.51)	1.82	3.45	(1.76 , 1.88)	(3.39 , 3.51)
	15 d - < 1 yr	1.55	2.76	(1.49 , 1.61)	(2.70 , 2.82)	1.55	2.76	(1.49 , 1.61)	(2.70 , 2.82)
	1 - < 5 yrs	1.39	2.21	(1.33 , 1.45)	(2.15 , 2.27)	1.39	2.21	(1.33 , 1.45)	(2.15 , 2.27)
	5 - < 13 yrs	1.33	1.94	(1.27 , 1.39)	(1.88 , 2.00)	1.33	1.94	(1.27 , 1.39)	(1.88 , 2.00)
	13 - < 16 yrs	1.02	1.81	(0.96 , 1.08)	(1.75 , 1.87)	1.14	2.01	(1.08 , 1.20)	(1.95 , 2.07)
	16 - < 19 yrs	0.95	1.63	(0.89 , 1.01)	(1.57 , 1.69)	0.95	1.63	(0.89 , 1.01)	(1.57 , 1.69)
Urea (mmol/L)	0 - < 14 d	1.0	8.7	(0.3 , 1.6)	(8.0 , 9.3)	1.0	8.7	(0.3 , 1.6)	(8.0 , 9.3)
	15 d - < 1 yr	1.2	6.3	(0.5 , 1.8)	(5.7 , 7.0)	1.2	6.3	(0.5 , 1.8)	(5.7 , 7.0)
	1 - < 10 yrs	3.3	8.4	(2.7 , 4.0)	(7.7 , 9.0)	3.3	8.4	(2.7 , 4.0)	(7.7 , 9.0)
	10 - < 19 yrs	2.7	7.2	(2.0 , 3.3)	(6.5 , 7.8)	2.7	7.9	(2.0 , 3.3)	(7.3 , 8.6)
Uric Acid (umol/L)	0 - < 14 d	165	718	(148, 182)	(700, 735)	165	718	(148, 182)	(700, 735)
	15 d - < 1 yr	100	364	(83, 117)	(347, 381)	100	364	(83, 117)	(347, 381)
	1 - < 12 yrs	111	282	(94, 128)	(265, 299)	111	282	(94, 128)	(265, 299)
	12 - < 19 yrs	155	338	(138, 172)	(321, 355)	158	435	(141, 175)	(418, 452)
Enzymes									
Amylase (U/L)	0 - 14 d	4	12	(<3 , 9)	(7 , 17)	4	12	(<3 , 9)	(7 , 17)
	15 d - <13ks	<3	25	(<3 , 8)	(20 , 30)	<3	25	(<3 , 8)	(20 , 30)
	13wks-<1yr	4	56	(<3 , 9)	(51 , 61)	4	56	(<3 , 9)	(51 , 61)
	1 - < 19 yrs	29	113	(24 , 33)	(108 , 118)	29	113	(24 , 33)	(108 , 118)
GGT[b] (U/L)	0 - 14 d	17	171	(12 , 22)	(166 , 176)	17	171	(12 , 22)	(166 , 176)
	15 d - < 1 yr	5	99	(<3 , 10)	(94 , 103)	5	99	(<3 , 10)	(94 , 103)
	1 - < 11 yrs	3	11	(<3 , 8)	(6 , 16)	3	11	(<3 , 8)	(6 , 16)
	11 - < 19 yrs	4	15	(<3 , 9)	(10 , 20)	4	15	(<3 , 9)	(10 , 20)
ALP (U/L)	0 - 14 d	89	260	(71,107)	(242 , 278)	89	260	(71,107)	(242 , 278)
	15 d - < 1 yr	130	490	(112,148)	(472 , 508)	130	490	(112,148)	(472 , 508)
	1 - < 10 yrs	150	350	(132,168)	(332 , 368)	150	350	(132,168)	(332 , 368)
	10 - < 13 yrs	136	435	(118,154)	(417 , 453)	136	435	(118,154)	(417 , 453)
	13 - < 15 yrs	62	267	(44 , 80)	(249 , 285)	123	489	(105 , 141)	(471 , 507)
	15 - < 17 yrs	55	124	(37 , 73)	(106 , 142)	88	346	(70 ,106)	(328 ,364)
	17 - < 19 yrs	50	93	(31 , 67)	(75 , 111)	60	158	(41 , 78)	(140 , 176)
ALT (U/L)	0 - < 1 yrs	8	31	(0 , 16)	(24 , 39)	8	31	(0 , 16)	(24 , 39)
	1 - < 13 yrs	11	25	(4 , 19)	(17 , 32)	11	25	(4 , 19)	(17 , 32)
	13 - < 19 yrs	11	22	(3 , 18)	(15 , 30)	11	24	(4 , 19)	(16 , 31)
ALT PP (U/L)	0 - < 1 yrs	7	36	(<4 , 17)	(26 , 45)	7	36	(<4 , 17)	(26 , 45)
	1 - < 13 yrs	12	28	(<4 , 21)	(18 , 37)	12	28	(<4 , 21)	(18 , 37)
	13 - < 19 yrs	10	25	(<4 , 20)	(15 , 34)	12	27	(<4 , 21)	(17 , 36)

(*continued on next page*)

Table 2 (*continued*)

AST (U/L)	0 - 14 d	33	154	(27 , 40)	(147 , 161)	33	154	(27 , 40)	(147 , 161)
	15 d - < 1 yr	22	66	(15 , 29)	(59 , 73)	22	66	(15 , 29)	(59 , 73)
	1 - < 7 yrs	23	44	(16 , 30)	(38 , 51)	23	44	(16 , 30)	(38 , 51)
	7 - < 12 yrs	20	37	(14 , 27)	(30 , 44)	20	37	(14 , 27)	(30 , 44)
	12 - < 19 yrs	16	28	(9 , 23)	(21 , 35)	17	36	(10 , 23)	(29 , 43)
AST PP (U/L)	0 - 14 d	40	175	(32 , 49)	(167 ,184)	40	175	(32 , 49)	(167 ,184)
	15 d - < 1 yr	28	77	(20 , 36)	(69 , 85)	28	77	(20 , 36)	(69 , 85)
	1 - < 7 yrs	29	53	(21 , 37)	(45 , 61)	29	53	(21 , 37)	(45 , 61)
	7 - < 12 yrs	26	45	(18 , 34)	(36 , 53)	26	45	(18 , 34)	(36 , 53)
	12 - < 19 yrs	21	34	(12 , 29)	(26 , 42)	22	44	(14 , 30)	(35 , 52)
LD (U/L)	0 - 14 d	303	1143	(251 , 356)	(1091 , 1196)	303	1143	(251 , 356)	(1091 , 1196)
	15 d - < 1 yr	169	435	(116 , 221)	(382 , 487)	169	435	(116 , 221)	(382 , 487)
	1 - < 10 yrs	196	314	(143 , 248)	(262 , 367)	196	314	(143 , 248)	(262 , 367)
	10 - < 15 yrs	163	269	(111 , 216)	(217 , 322)	175	279	(123 , 228)	(227 , 332)
	15 - < 19 yrs	139	249	(86 , 191)	(196 , 301)	139	249	(86 , 191)	(196 , 301)
Proteins									
Albumin (BCP) (g/L)	0 - 14 d	28	41	(25 , 30)	(39 , 44)	28	41	(25 , 30)	(39 , 44)
	15 d - < 1 yr	24	47	(22 , 27)	(44 , 49)	24	47	(22 , 27)	(44 , 49)
	1 - < 8 yrs	35	46	(33 , 37)	(43 , 48)	35	46	(33 , 37)	(43 , 48)
	8 - < 15 yrs	37	48	(35 , 40)	(45 , 50)	37	48	(35 , 40)	(45 , 50)
	15 - < 19 yrs	35	50	(33 , 37)	(48 , 52)	38	51	(36 , 41)	(49 , 53)
Albumin (BCG) (g/L)	0 - 14 d	33	45	(31 , 36)	(43 , 47)	33	45	(31 , 36)	(43 , 47)
	15 d - < 1 yr	31	50	(29 , 33)	(48 , 52)	31	50	(29 , 33)	(48 , 52)
	1 - < 8 yrs	40	49	(38 , 42)	(47 , 51)	40	49	(38 , 42)	(47 , 51)
	8 - < 15 yrs	42	51	(40 , 44)	(49 , 53)	42	51	(40 , 44)	(49 , 53)
	15 - < 19 yrs	40	53	(38 , 42)	(51 , 55)	43	53	(41 , 45)	(51 , 56)
ASO (IU/mL)	0 - < 6 mths	<20[c]	<20	(<20 , <20)	(<20 , <20)	<20	<20	(<20 , <20)	(<20 , <20)
	6 mths-<1yr	<20	<20	(<20 , <20)	(<20 , 66)	<20	<20	(<20 , <20)	(<20 , 66)
	1 - < 6 yrs	<20	134	(<20 , <20)	(76 , 192)	<20	134	(<20 , <20)	(76 , 192)
	6 - < 19 yrs	<20	518	(<20 , <20)	(461 ,576)	<20	518	(<20 , <20)	(461 ,576)
C3 (g/L)	0 - 14 d	0.52	1.26	(0.42 , 0.62)	(1.16 , 1.36)	0.52	1.26	(0.42 , 0.62)	(1.16 , 1.36)
	15 d - < 1 yr	0.53	1.67	(0.43 , 0.63)	(1.57 , 1.77)	0.53	1.67	(0.43 , 0.63)	(1.57 , 1.77)
	1 - < 19 yrs	0.86	1.59	(0.76 , 0.96)	(1.49 , 1.69)	0.86	1.59	(0.76 , 0.96)	(1.49 , 1.69)
C4 (g/L)	0 - < 1 yr	0.07	0.30	(0.05 , 0.09)	(0.28 , 0.32)	0.07	0.30	(0.05 , 0.09)	(0.28 , 0.32)
	1 - < 19 yrs	0.13	0.37	(0.11 , 0.15)	(0.35 , 0.39)	0.13	0.37	(0.11 , 0.15)	(0.35 , 0.39)
CRP (mg/L)	0 - 14 d	0.0	5.4	(0.0 , 1.7)	(3.4 , 7.4)	0.0	5.4	(0.0 , 1.7)	(3.4 , 7.4)
	15d -<15yrs	0.0	0.4	(0.0 , 1.5)	(0.0 , 2.4)	0.0	0.4	(0.0 , 1.5)	(0.0 , 2.4)
	15 - < 19 yrs	0.0	1.1	(0.0 , 1.5)	(0.0 , 3.0)	0.0	1.1	(0.0 , 1.5)	(0.0 , 3.0)
Haptoglobin (g/L)	0 - 14 d	<0.10	0.11	(<0.10 , 0.17)	(<0.10 , 0.26)	<0.10	0.11	(<0.10 , 0.17)	(<0.10 , 0.26)
	15 d - < 1 yr	<0.10	2.09	(<0.10 , 0.24)	(1.94 , 2.24)	<0.10	2.09	(<0.10 , 0.24)	(1.94 , 2.24)
	1 - < 12 yrs	<0.10	1.54	(<0.10 , 0.24)	(1.39 , 1.69)	<0.10	1.54	(<0.10 , 0.24)	(1.39 , 1.69)
	12 - < 19 yrs	<0.10	1.69	(<0.10 , 0.24)	(1.54 , 1.84)	<0.10	1.69	(<0.10 , 0.24)	(1.54 , 1.84)
IgA (g/L)	0 - < 1 yrs	<0.1	0.2	(<0.1 , <0.1)	(<0.1 , 0.4)	<0.1	0.2	(<0.1 , <0.1)	(<0.1 , 0.4)
	1 - < 3 yrs	<0.1	0.8	(<0.1 , <0.1)	(0.6 , 1.1)	<0.1	0.8	(<0.1 , <0.1)	(0.6 , 1.1)
	3 - < 6 yrs	0.2	1.5	(<0.1 , 0.4)	(1.3 , 1.7)	0.2	1.5	(<0.1 , 0.4)	(1.3 , 1.7)
	6 - < 14 yrs	0.4	2.3	(0.2 , 0.6)	(2.1 , 2.5)	0.4	2.3	(0.2 , 0.6)	(2.1 , 2.5)
	14 - < 19 yrs	0.4	3.1	(0.2 , 0.6)	(2.9 , 3.3)	0.4	3.1	(0.2 , 0.6)	(2.9 , 3.3)
IgG (g/L)	0 - 14 d	3.3	13.2	(2.7 , 3.8)	(12.7 , 13.7)	3.3	13.2	(2.7 , 3.8)	(12.7 , 13.7)
	15 d - < 1 yr	1.3	6.8	(0.8 , 1.9)	(6.2 , 7.3)	1.3	6.8	(0.8 , 1.9)	(6.2 , 7.3)
	1 - < 4 yrs	3.3	10.9	(2.7 , 3.8)	(10.4 , 11.4)	3.3	10.9	(2.7 , 3.8)	(10.4 , 11.4)
	4 - < 10 yrs	5.3	12.8	(4.8 , 5.8)	(12.3 , 13.4)	5.3	12.8	(4.8 , 5.8)	(12.3 , 13.4)
	10 - < 19 yrs	6.4	14.4	(5.9 , 6.9)	(13.9 , 14.9)	6.4	14.4	(5.9 , 6.9)	(13.9 , 14.9)
IgM (g/L)	0 - 14 d	0.1	0.4	(<0.1 , 0.2)	(0.2 , 0.5)	0.1	0.4	(<0.1 , 0.2)	(0.2 , 0.5)
	15d-<13wks	0.1	0.7	(<0.1 , 0.2)	(0.6 , 0.9)	0.1	0.7	(<0.1 , 0.2)	(0.6 , 0.9)
	13wks -<1yr	0.2	0.9	(<0.1 , 0.3)	(0.8 , 1.1)	0.2	0.9	(<0.1 , 0.3)	(0.8 , 1.1)
	1 - < 19 yrs	0.5	2.0	(0.4 , 0.7)	(1.8 , 2.1)	0.4	1.6	(0.2 , 0.5)	(1.4 , 1.7)
Total Protein (g/L)	0 - 14 d	55	83	(53 , 57)	(81 , 85)	55	83	(53 , 57)	(81 , 85)
	15 d - < 1 yr	46	72	(44 , 48)	(70 , 74)	46	72	(44 , 48)	(70 , 74)
	1 - < 6 yrs	62	76	(60 , 64)	(74 , 78)	62	76	(60 , 64)	(74 , 78)
	6 - < 9 yrs	65	77	(63 , 67)	(76 , 80)	65	77	(63 , 67)	(76 , 80)
	9 - < 19 yrs	66	81	(64 , 68)	(80 , 83)	66	81	(64 , 68)	(80 , 83)

Table 2 (*continued*)

Transferrin (g/L)	0 - < 9 wks	1.15	2.44	(0.99, 1.31)	(2.28, 2.60)	1.15	2.44	(0.99, 1.31)	(2.28, 2.60)
	9 wks - <1yr	1.18	3.52	(1.02, 1.34)	(3.36, 3.68)	1.18	3.52	(1.02, 1.34)	(3.36, 3.68)
	1 - < 19 yrs	2.40	3.66	(2.24, 2.56)	(3.50, 3.82)	2.40	3.66	(2.24, 2.56)	(3.50, 3.82)
Lipids/Lipoproteins									
ApoB (g/L)	0 - 14 d	<0.0	1.2	(<0.0 , 0.1)	(1.1 , 1.3)	<0.0	1.2	(<0.0 , 0.1)	(1.1 , 1.3)
	15 d - < 1 yr	0.2	2.4	(0.1 , 0.3)	(2.2 , 2.5)	0.2	2.4	(0.1 , 0.3)	(2.2 , 2.5)
	1 - < 6 yrs	0.7	1.7	(0.5 , 0.8)	(1.6 , 1.9)	0.7	1.7	(0.5 , 0.8)	(1.6 , 1.9)
	6 - < 19 yrs	0.5	1.6	(0.3 , 0.6)	(1.4 , 1.7)	0.5	1.6	(0.3 , 0.6)	(1.4 , 1.7)
Cholesterol (mmol/L)	0 - 14 d	1.29	3.38	(1.07 , 1.51)	(3.16 , 3.60)	1.19	2.96	(0.97 , 1.41)	(2.74 , 3.18)
	15 d - < 1 yr	1.76	6.37	(1.54 , 1.98)	(6.15 , 6.59)	1.76	6.37	(1.54 , 1.98)	(6.15 , 6.59)
	1 - < 19 yrs	3.04	5.62	(2.82 , 3.26)	(5.40 , 5.84)	3.04	5.62	(2.82 , 3.26)	(5.40 , 5.84)
HDLC (mmol/L)	0 - 14 d	0.22	1.00	(0.09 , 0.35)	(0.87 , 1.13)	0.22	1.00	(0.09 , 0.35)	(0.87 , 1.13)
	15 d - < 1 yr	0.10	1.89	(<0.08 , 0.23)	(1.76 , 2.02)	0.10	1.89	(<0.08 , 0.23)	(1.76 , 2.02)
	1 - < 4 yrs	0.72	1.63	(0.59 , 0.85)	(1.50 , 1.76)	0.72	1.63	(0.59 , 0.85)	(1.50 , 1.76)
	4 - < 13 yrs	0.82	1.92	(0.69 , 0.95)	(1.79 , 2.05)	0.82	1.92	(0.69 , 0.95)	(1.79 , 2.05)
	13 - < 19 yrs	0.71	1.90	(0.58 , 0.84)	(1.77 , 2.03)	0.70	1.79	(0.57 , 0.83)	(1.66 , 1.92)
Triglycerides (mmol/L)	0 - 14 d	0.98	3.21	(0.75 , 1.21)	(2.98 , 3.44)	0.98	3.21	(0.75 , 1.21)	(2.98 , 3.44)
	15 d - < 1 yr	0.61	3.20	(0.38 , 0.84)	(2.97 , 3.43)	0.61	3.20	(0.38 , 0.84)	(2.97 , 3.43)
	1 - < 19yrs	0.50	2.43	(0.27 , 0.73)	(2.20 , 2.66)	0.50	2.43	(0.27 , 0.73)	(2.20 , 2.66)

[a]Partitions requiring sex partitioning are underlined. Female reference intervals are highlighted in pink, whereas male reference intervals are highlighted in blue.

[b]GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ALT PP, alanine aminotransferase with pyridoxal phosphate; AST, aspartate aminotransferase; AST PP, aspartate aminotransferase with pyridoxal phosphate; LD, lactate dehydrogenase; Albumin BCP, albumin bromcresol purple; Albumin BCG, albumin bromcresol green; ASO, anti-streptolysin O; C3, complement C3; C4, complement C4; CRP, C-reactive protein; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; ApoB, apolipoprotein B; HDLC, high-density lipoprotein cholesterol.

[c]'<' indicates values lower than the detection limit

transferable. However, further analysis was needed to assess transference of the reference intervals for the hsCRP assay to the cobas 6000 due to failure to meet criteria of the normality plots initially (see Fig. 2B–D), despite a strong correlation between the two systems with an $R^2 = 0.926$ (See Fig. 2A). Therefore, reference intervals for the CRP assay on the Roche cobas 6000 are unable to be provided. However, for hsCRP specifically we decided to remove values >25 mg/L, as hsCRP is most commonly used to assess cardiovascular disease risk with levels > 3.0 mg/L indicative of high risk, rather than infection/inflammation which can cause levels to increase up to 200 mg/L. Within this narrower range, more relevant to hsCRP, the correlation improved to $R^2 = 0.992$ (see Fig. 3A). In addition to a stronger positive correlation, the normality plots also improved (see Fig. 3B–D), which therefore allowed the hsCRP assay to be transferred from the Abbott Architect to the Roche cobas 6000. After assessing statistical assumptions, 13 of 16 (81%) assays were transferable to the cobas 6000, and 31 of 36 (86%) assays were transferrable to the Modular P.

3.2. Verification of transferred reference intervals

To verify the transferred reference intervals, 100 reference samples from the healthy CALIPER pediatric cohort [2] were analyzed. Table 3 summarizes the assays that were verified (total verification across all partitions) for the cobas 6000 and the Modular P. Verification was considered successful if ≥90% of values fell within the reference limits, inclusive of the 95% confidence intervals. Based on this criteria, 62% (8 of 13) of transferable analytes were verified for the cobas 6000 and 61% (19 of 31) were verified for the Modular P. In contrast, if only the upper and lower limits of the reference intervals were considered for verification (as per CLSI guidelines) [1], excluding the 95% confidence intervals, only 23% and 16% of assays would be considered verified on the cobas 6000 and Modular P, respectively. When the verification cut-off was lowered to 80%, while still including the 95% confidence intervals, the percentage of transferable assays increased to 92% (12 of 13) for the cobas 6000 and 90% (28 of 31) for the Modular P.

4. Discussion

In the present study, analytes assayed on the Abbott Architect were compared to corresponding assays on the Roche cobas 6000 and the Modular P, to transfer and verify CALIPER pediatric reference intervals to Roche assays. The transference and verification protocols closely followed CLSI guidelines with one minor exception. Whereas CLSI guidelines recommend a minimum of 20 independent patient samples, we used 200 pooled serum specimens to derive the linear equation for transference and an additional 100 healthy reference samples from the CALIPER biobank to verify the reference intervals. Furthermore, we examined normality and the absence of relative or absolute data clustering to ensure a valid linear relationship between methods. We also used Deming regression when $R^2 < 0.95$ to account for variability in both methods, and thus more accurately represent the correlation [12]. Therefore, the CALIPER transference protocol is much more rigorous than the requirements of CLSI, and accordingly, we concluded that it was appropriate to use the reference intervals inclusive of the 95% confidence intervals as a defining factor for verification. It is important to note that assay comparisons performed here make no assumption as to which system is more accurate, as differences may exist in assay calibration and other parameters.

Overall, most assays were strongly correlated between the Abbott and Roche methods. The assay-specific, age- and sex-stratified reference intervals for Roche assays (Tables 1–2) are similar to those established in our initial CALIPER study using Abbott assays [2]. For some analytes, the transferred reference intervals for all partitions fell entirely within the 90% confidence interval previously established by CALIPER. These include C4 on both instruments, apoB on the cobas 6000, and phosphate, uric acid, ALP, ALT, LD, IgG, and total protein on the Modular P. Although the reference intervals were comparable between platforms, for many of the other tests, the transferred reference intervals had at least one partition where the lower and/or upper reference limits fell outside the 90% confidence intervals of the corresponding Abbott reference limit established previously [2].

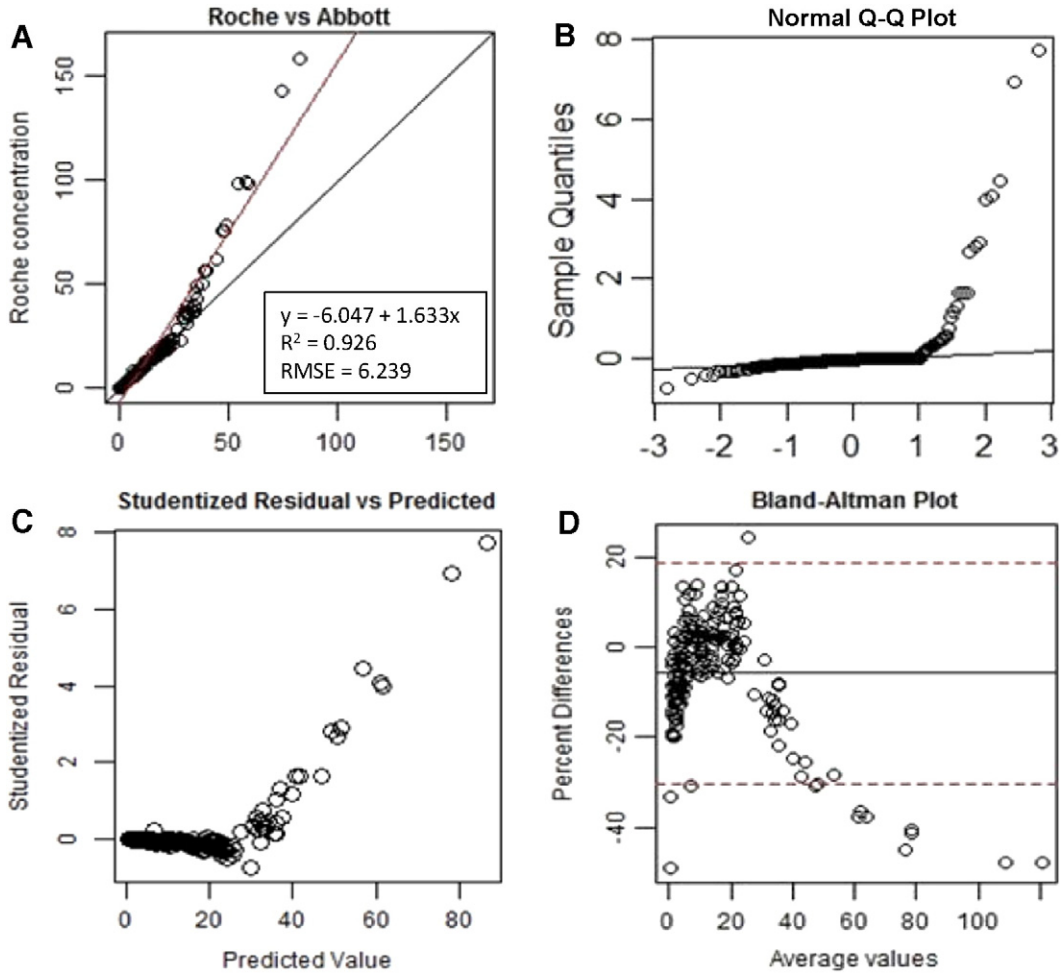

Fig. 2. Statistical criteria used to assess the appropriateness of transference. Scatter (A), Q–Q (B), studentized residual (C) and Bland–Altman (D) plots for high sensitivity C-reactive protein (hsCRP) on Roche cobas 6000. Scatter plot indicates strong positive correlation, while normality plots indicate that transference is inappropriate for dataset. In the scatterplot (A), the line of perfect agreement is shown in black, while the regression line is shown in red.

The most extreme example of an assay-specific difference was observed for ASO on both Roche instruments, where results obtained using Roche assays were approximately 30% and 60% higher for the 1 to <6 and 6 to <19 year age partitions, respectively, compared to values obtained with the Abbott assay. Another CALIPER transference study between Abbott and the Beckman Coulter DxC800 also showed a notable difference between the ASO assays 13. Abbott uses the Rantz–Randall (hemolytic) method, a semi-quantitative measurement of the inhibition of Streptolysin-O-induced hemolysis of erythrocytes by ASO [14]. In contrast, Roche platforms use an immunoturbidometric assay [14]. Hence, methodology differences may explain the dissimilarities observed in the calculated reference intervals between these platforms. Although this variation seems substantial, it is much less extreme than examples previously noted in the CALIPER transference study by Estey et al., in which lipase was approximately 4-fold higher using the Siemens Vista assay compared to the Abbott assay [7].

The select analytes that did not meet the statistical requirements for transference to the Roche instruments are of key interest. Both bicarbonate and magnesium did not transfer from Abbott to either Roche assays due to poor correlation ($R^2 < 0.70$). A similar trend was observed in a previous CALIPER transference study, however, the methodology was similar between the Abbott and Beckman instruments [7] indicating that other sources of variation such as pre-analytical factors provide a likely explanation. Carbon dioxide is volatile and can easily escape from the sample during storage, transport, and aerobic handling [15]. The magnesium assay, however, likely failed to transfer due to methodology differences. The Roche method is a dye binding assay [16], whereas the Abbott method is an enzymatic assay [17].

Pediatric reference intervals for two additional assays, total calcium ($R^2 = 0.53$) and prealbumin ($R^2 = 0.27$), failed to transfer to the Roche Modular P because of poor correlation. Both Abbott and Roche measure the concentration of total calcium using colorimetric assays. However, the two methods use different dyes with each chromophore's peak absorbance at a different wavelength: Abbott Architect uses arsenazo III dye binding [18], while the Modular P uses 5-nitro-5′-methyl-BAPTA. These results are not surprising as total calcium also failed to transfer to the Beckman Coulter DxC800 and AU, in other CALIPER transference studies [13,19]. Regarding prealbumin, both Abbott and Roche assays use an immunoturbidometric assay for measurement, however the calibration reference materials are different. Abbott uses a WHO International Standard Preparation of Rheumatoid Arthritis Serum (NIBSC code 64/2), while the Modular P method is studentized against Institute for Reference Materials and Measurements (IRMM) certified reference material (ERM-DA470k/IFCC).

Another analyte of interest is CRP, which initially failed to meet additional statistical criteria for transference to the cobas 6000 assay, although a high correlation ($R^2 = 0.926$) was demonstrated. hsCRP and CRP measurements are used clinically as a cardiac marker and as a stress and/or infection marker, respectively. In response to stress and

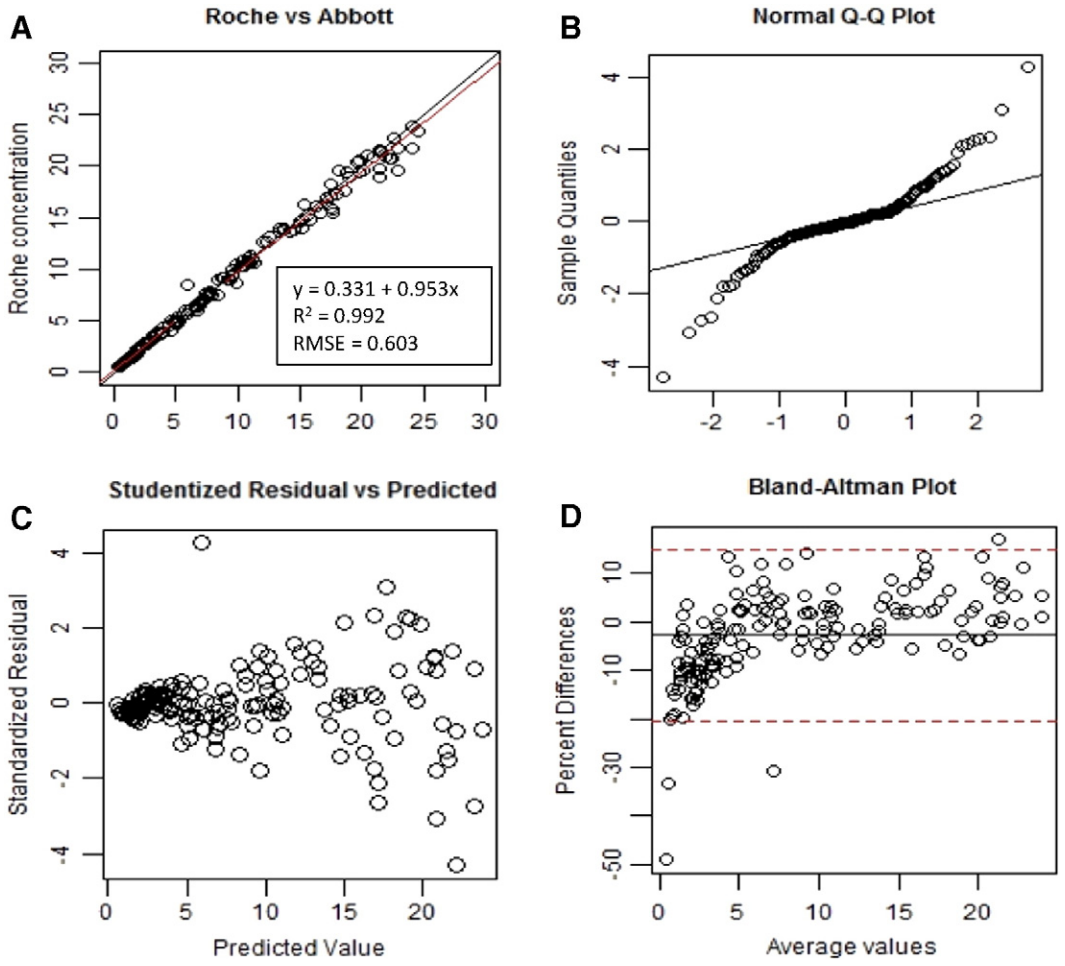

Fig. 3. Statistical criteria used to assess the appropriateness of transference. Scatter (A), Q–Q (B), studentized residual (C) and Bland–Altman (D) plots for high sensitivity C-reactive protein (hsCRP) on Roche cobas 6000 after removal of data points above 25 mg/L. All plots indicate that transference is appropriate. In the scatterplot (A), the line of perfect agreement is shown in black, while the regression line is shown in red.

infection, CRP can fluctuate widely and has extremely high values [20], while very high values are not of practical interest for hsCRP. When observing the entire dataset, CRP was not transferable to the cobas 6000 due to failure to meet additional statistical criteria and therefore CRP reference intervals specific for the cobas 6000 could not be reported. For hsCRP specifically, values >25 mg/L are not of practical interest. Thus, when these values were removed from the data set, the hsCRP assay was able to be transferred to the Roche cobas 6000 with a very strong correlation ($R^2 = 0.992$) and the ability to meet additional statistical criteria (see Fig. 3).

An important aspect of transference studies is an objective approach to verify transferred intervals using patient samples that span the linear range. Using 100 CALIPER samples, the transferred reference intervals were verified on the basis that >90% of the reference samples should fall within the 95% confidence intervals of the transferred reference intervals. Based on this criteria, 62% (8 of 13) of reference intervals for transferable assays were verified for the cobas 6000 and 61% (19 of 31) were verified for the Modular P. The majority of the assays that did not fall within the reference interval fell just outside the boundaries, which is demonstrated by the fact that when only the reference limits are considered for verification, only 23% and 16% of assays would be considered verified on the cobas 6000 and Modular P, respectively. The percentage of transferable assays increased to 92% (12 of 13) for the cobas 6000 and 90% (28 of 31) for the Modular P when the verification cut-off was lowered to 80%, while still including the 95% confidence intervals, demonstrating that many assays that failed verification only failed by a slight margin.

As these reference intervals were determined through transference, it is important to compare them to reference intervals established from full reference interval studies on these analytical platforms. A group from the United States used the Roche Modular P analyzer to measure 7 serum analytes in 1765 healthy children aged 6 months to 17 years [9]. The amylase reference interval established for the 6 month to 2 year interval was greater than that established for our 13 week to 1 year interval (12–113 U/L vs. 4–56 U/L), however the difference in age partitions does make it difficult to directly compare. For older children and adolescents however, the reference intervals were very similar. Clifford et al. used predetermined age partitions and thus from 3 to 17 years, 5 reference intervals were established with the lower limit ranging from 26–31 U/L and upper limit ranging from 112–163 U/L. The reference interval we determined through transference was very similar: 29–113 U/L for 1 to 19 years. Uric acid reference intervals were relatively similar between studies, however our values were lower for older children and adolescents and wider for the young age partition. The 6 month to 2 year age partition established by Clifford et al. was 131–333 U/L, whereas ours was 100–364 U/L for infants aged 15 day to 1 year. A greater variation in uric acid levels can be expected in the neonatal time period [2], contributing to the wider reference interval established in this study as our partition includes this early age period.

Kulasingam et al. established reference intervals for 28 analytes on the Roche cobas 6000 using samples from approximately 600 outpatients (aged 0 to 20 years) who were deemed to be metabolically stable [8]. The creatinine reference intervals established by Kulasingam et al.

Table 3
Percentage of CALIPER samples that fell within transferred reference intervals inclusive of 95% confidence limits (exclusive of 95% confidence limits in parentheses).[a]

	Analyte	Roche cobas 6000	Roche Modular P
Chemistry	Bilirubin Direct	90.7 (86.6)	92.8 (64)
	Bilirubin Total	ND[b]	91.1 (81.2)
	Calcium	ND	NT[c]
	Bicarbonate (HCO_3^-)	NT	NT
	Creatinine	59.8 (45.4)	58 (35.7)
	Iron	ND	92 (88.4)
	Magnesium	NT	NT
	Phosphate	ND	87.5 (76.8)
	Urea	ND	96.4 (90.2)
	Uric Acid	ND	86.6 (83.9)
Enzymes	Amylase	90.7 (87.6)	88.4 (86.6)
	Gamma Glutamyl Transferase (GGT)	88.7 (72.2)	92 (76.8)
	Alkaline Phosphatase (ALP)	ND	67 (64.3)
	Aspartate Aminotransferase (AST)	ND	91.1 (67)
	AST with Pyridoxal Phosphate (AST PP)	ND	93.8 (67.9)
	Alanine Aminotransferase (ALT)	ND	96.4 (76.8)
	ALT with Pyridoxal Phosphate (ALT PP)	ND	95.5 (87.5)
	Lactate Dehydrogenase (LD)	ND	93.8 (61.6)
Proteins	Albumin Bromcresol Green (BCG)	99 (95.9)	95.5 (87.5)
	Albumin Bromcresol Purple (BCP)	ND	99.1 (92.9)
	Antistreptolysin-O (ASO)	86.6 (82.5)	84.7 (74.8)
	C reactive Protein (CRP)	NT	86.6 (54.5)
	Complement C3 (C3)	99 (96.9)	97.3 (91.1)
	Complement C4 (C4)	91.8 (83.5)	94.6 (85.7)
	Haptoglobin	ND	97.3 (86.5)
	High sensitivity C reactive Protein (hsCRP)	86.6 (51.5)	ND
	Immunoglobulin A (IgA)	ND	76.8 (70.7)
	Immunoglobulin G (IgG)	ND	87.6 (78.4)
	Immunoglobulin M (IgM)	ND	89.1 (80.4)
	Protein Total	ND	85.7 (79.5)
	Transferrin	96.9 (86.6)	95.5 (92.9)
	PreAlbumin	ND	NT
	Rheumatoid Factor (RF)	NT	NT
Lipids/Lipoproteins	Apolipoprotein B (Apo B)	88.7 (80.4)	96.4 (88.4)
	Cholesterol Total	ND	96.4 (96.4)
	High-density lipoprotein cholesterol (HDLC)	94.8 (93.8)	88.8 (86.9)
	Triglyceride Total	92.8 (86.6)	95.5 (89.3)

[a]The percentage of CALIPER samples (n=100) that fell within the appropriate partitioned reference interval inclusive of the 95% confidence intervals is shown for each assay. The number in parentheses represents the percentage of samples that fell within the appropriate partitioned reference interval excluding the 95% confidence intervals. Results highlighted in green indicate that the reference interval was verified, defined as >90% of CALIPER samples falling within the reference interval inclusive of the 95% confidence interval. In cases where < 90% of the CALIPER samples fell within the transferred reference intervals, inclusive of the 95% confidence intervals, the result is highlighted in pink (reference interval not verified).

[b]ND, not determined.

[c]NT, not transferable based on statistical criteria.

were very similar across the entire age range to those established by our current transference study. For example, their 0 to <1 year reference interval was 15–37 μmol/L, while our 15 day to <2 year reference interval was 15–38 μmol/L. The apoB reference intervals were also very similar: 0.40–0.94 g/L and 0.29–0.85 g/L for 1 to <6 years and 6 to <19 years, respectively, compared to 0.38–1.05 g/L for 1 to <20 years established by the full reference interval study [8]. Additionally, reference intervals for triglycerides were very comparable: 0.54–2.47 mmol/L for 1 to

<19 years compared to 0.43–2.75 mmol/L for 1 to <20 years established by Kulasingam et al. Lastly, reference intervals for direct bilirubin were similar between studies. The lower limit was below the detection limit for all age partitions in both studies, except for the 0 to 14 day partition in the current transference study. The upper limits were also similar: 2.43 μmol/L for the 1 to <9 year age partition established in the current study and 1.90 and 2.87 μmol/L for the 1 to <5 year and 5 to <10 year partitions, respectively [8].

In conclusion, the major advantage of using transference to determine reference intervals for a new analytical system is that it removes the need to collect and test samples from a large number of healthy reference individuals for each partition [1]. The data presented in this study supports the use of the transference approach as an effective method to generate reference intervals for many assays. This CALIPER study established the relationship between Abbott Architect assays and two other commonly used analytical platforms, the Roche cobas 6000 and the Roche Modular P, to successfully transfer age- and sex-specific pediatric reference intervals previously established by CALIPER [2]. These new pediatric reference intervals expand the utility of the CALIPER database and facilitate their broader application to pediatric centers using Roche assays. It is important to note however that this study provides transference data for specific assays, and does not verify reference intervals for analyzers at other laboratories, specific populations, or geographic locations other than the population used in this study (the GTA and Hamilton region). Each laboratory must verify reference intervals to ensure accuracy and specificity for their specific analytical system, local population and geographic location.

Establishing common reference intervals across all analytical platforms and in all pediatric centers across Canada to facilitate harmonization is a future goal of CALIPER [21]. The transferred CALIPER reference intervals for different analytical platforms from this study as well as the previous ones [7,13,19] can later be collectively analyzed to develop common reference intervals that will be applicable to assays across different analytical platforms, helping to harmonize test interpretation in clinical laboratories worldwide.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.clinbiochem.2015.08.018.

Acknowledgments

This study was supported by an operating grant from the Canadian Institutes of Health Research (CIHR), grant number (201103MOP-246968) and Roche Diagnostics Canada. VH was supported by a Canada Graduate Scholarship (CGS) Master's Award and a Banting and Best Diabetes Centre (BBDC) Novo Nordisk Studentship. We appreciate the participation of all of the CALIPER participants and their families, as well as the CALIPER coordinators and volunteers.

References

[1] Clinical and Laboratory Standards Institute (CLSI), Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline, CLSI document C28-A3, third edition, 2008.
[2] D.A. Colantonio, L. Kyriakopoulou, M.K. Chan, C.H. Daly, D. Brinc, A.A. Venner, et al., Closing the gaps in pediatric laboratory reference intervals: a CALIPER database of 40 biochemical markers in a healthy and multiethnic population of children, Clin. Chem. 58 (2012) 854–868.
[3] S. Mansoub, M.K. Chan, K. Adeli, Gap analysis of pediatric reference intervals for risk biomarkers of cardiovascular disease and the metabolic syndrome, Clin. Biochem. 39 (2006) 569–587.
[4] K. Adeli, Closing the gaps in pediatric reference intervals: the CALIPER initiative, Clin. Biochem. 44 (2011) 480–482.
[5] D. Konforte, J.L. Shea, L. Kyriakopoulou, D. Colantonio, A.H. Cohen, J. Shaw, et al., Complex biological pattern of fertility hormones in children and adolescents: a study of healthy children from the CALIPER cohort and establishment of pediatric reference intervals, Clin. Chem. 59 (2013) 1215–1227.
[6] V. Bevilacqua, M.K. Chan, Y. Chen, D. Armbruster, B. Schodin, K. Adeli, Pediatric population reference value distributions for cancer biomarkers and covariate-stratified reference intervals in the CALIPER cohort, Clin. Chem. 60 (2014) 1532–1542.
[7] M.P. Estey, A.H. Cohen, D.A. Colantonio, M.K. Chan, T.B. Marvasti, E. Randell, et al., CLSI-based transference of the CALIPER database of pediatric reference intervals from Abbott to Beckman, Ortho, Roche and Siemens clinical chemistry assays: direct validation using reference samples from the CALIPER cohort, Clin. Biochem. 46 (2013) 1197–1219.
[8] V. Kulasingam, B.P. Jung, I.M. Blasutig, S. Baradaran, M.K. Chan, M. Aytekin, et al., Pediatric reference intervals for 28 chemistries and immunoassays on the Roche cobas 6000 analyzer—a CALIPER pilot study, Clin. Biochem. 43 (2010) 1045–1050.
[9] S.M. Clifford, A.M. Bunker, J.R. Jacobsen, W.L. Roberts, Age and gender specific pediatric reference intervals for aldolase, amylase, ceruloplasmin, creatine kinase, pancreatic amylase, prealbumin, and uric acid, Clin. Chim. Acta 412 (2011) 788–790.
[10] CLSI, Method comparison and bias estimation using patient samples: approved guideline, CLSI document EP9-A22nd ed., 2002.
[11] R Core Team, A language and environment for statistical computing. , R Foundation for Statistical Computing, 2013 (Ref Type: Online Source (http://web.mit.edu/r_v3.0.1/fullrefman.pdf (Accessed May 25, 2015))).
[12] R.F. Martin, General Deming regression for estimating systematic bias and its confidence interval in method-comparison studies, Clin. Chem. 46 (2000) 100–104.
[13] P.A. Araujo, D. Thomas, T. Sadeghieh, V. Bevilacqua, M.K. Chan, Y. Chen, et al., CLSI-based transference of the CALIPER database of pediatric reference intervals to Beckman Coulter DxC biochemical assays, Clin. Biochem. 2015 In Press [Epub ahead of print].
[14] W. Leowattana, A. Leelarasamee, P. Tobunluepop, S. Kiartivich, K. Sribhen, Normal range of serum anti-streptolysin-O determined by automated turbidimetric immunoassay, J. Infect. Dis. Antimicrob. Agents 61–5 (1997).
[15] Z.L. Bandi, Estimation, prevention, and quality control of carbon dioxide loss during aerobic sample processing, Clin. Chem. 27 (1981) 1676–1681.
[16] CSCL assay methodologies, Advanced Research and Diagnostic Laboratory — University of Minnesota, 2010 (Ref Type: Online Source (http://www.ardl.umn.edu/methodologies/home.html (Accessed May 25, 2015))).
[17] D. Bailey, P. Martens, W. Mah, P.M. Yip, A next generation enzymatic magnesium assay on the Abbott Architect chemistry system meets performance goals based on biological variation, Clin. Biochem. 47 (2014) 142–144.
[18] B.R. Morgan, J.D. Artiss, B. Zak, Calcium determination in serum with stable alkaline Arsenazo III and triglyceride clearing, Clin. Chem. 39 (1993) 1608–1612.
[19] M.A. El Hassan, A. Stoianov, P.A. Araujo, T. Sadeghieh, M.K. Chan, Y. Chen, et al., CLSI-based transference of CALIPER pediatric reference intervals to Beckman Coulter AU biochemical assays, Clin. Biochem. (2007) In Press [Epub ahead of print].
[20] J.J. Morley, I. Kushner, Serum C-reactive protein levels in disease, Ann. N. Y. Acad. Sci. 389 (1982) 406–418.
[21] J.L. Shaw, M.T. Binesh, D. Colantonio, K. Adeli, Pediatric reference intervals: challenges and recent initiatives, Crit. Rev. Clin. Lab. Sci. 50 (2013) 37–50.